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INTERACTIONS OF GIBBERELLIN, VERNALIZATION, PHOTOPERIOD AND TEMPERATURE IN THE FLOWERING OF ENDIVE^{1 2 3}

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Stem elongation and concomitant acceleration of flowering are well known responses of some higher plants to treatment with gibberellin (2, 3, 7, 8, 19, 20). It is clear that responsive biennials, such as henbane (*Hyoscyamus niger* L.) and carrot (*Daucus carota* L.) must reach a certain stage of development before either cold or gibberellin is effective in promoting seed-stalk elongation and flowering (4, 5, 9, 19). Thus, in carrot a minimum root diameter of about 1 cm must be attained before flowering may be induced (4, 19). The flowering behavior of the biennials is in contrast to that of winter annual plants such as head lettuce (*Lactuca sativa* L.) and endive (*Cichorium endivia* L.). The winter annuals, one of which is discussed in this paper, have only a quantitative requirement for cold. Flowering is hastened by a short period of seedling vernalization followed by a regimen of long days and high night temperatures (6, 10, 11, 12, 13, 14, 16, 18, 19). Application of gibberellin at any time after seed germination promotes stem growth and subsequent flowering of endive and of lettuce. The questions which the present experiments were designed to answer are: a) to what extent does gibberellin replace the cold and/or long day requirements for flowering of endive, and b) to what extent does gibberellin overcome temperatures unfavorable for seedstalk elongation.

METHODS

These studies were performed in the Earhart Plant Research Laboratories (17) between December 15, 1957, and September 1, 1958. All plants were grown in a 1:1 mixture of vermiculite and gravel and were supplied 3 times a week with a modified Hoagland's nutrient solution. The experiments were set up in a split-split-split plot design. The statistical analyses of numbers of days and stem heights at the appearance of the developing inflorescences were so conducted as to take into account the fact that non-vernalized

plants without gibberellin did not flower and hence could not be included in the analyses of those treatments which did flower. The index of growth response used was stem height at the time of appearance of the developing inflorescence. The number of leaves on the central axis below the developing inflorescence (11, 12, 13, 14) was used as a physiological index. Number of days to the appearance of the developing inflorescence was used as a chronological index. Each treatment of each experiment consisted of 5 to 16 replications, each of 1 plant. The exact numbers are detailed below. Variance ratios (F values) for main effects (temperature, photoperiod, vernalization and gibberellin) were all significant at the 1 % level for all variables measured except for the effect of temperature on days to flower (table IB) which was significant at the 5 % level.

RESULTS

In an initial experiment endive seedlings (variety broadleaf Batavian) were vernalized 20 days at 4° C. Six single plant replications of each treatment were then grown in greenhouses under either 8 or 16 hours respectively, of natural light or of natural light supplemented by low intensity artificial light (about 50 ft-c) from incandescent lamps. The temperatures were controlled at 23° C from 8 A.M. to 4 P.M. and at 10°, 17° or 23° C from 4 P.M. to 8 A.M. Non-vernalized control seed for all environmental conditions was planted 2 days before the vernalized seedlings were transferred from the 4° C room, thus providing seedlings (at the end of cold treatment) of approximately the same size and appearance. After expansion of the 2nd true leaf 50 or 100 µg of gibberellin per plant was applied to the vegetative apices of a portion of the plants under each growing condition. Only the data for the 50 µg treatments are presented since the 2 concentrations elicited similar responses. The results (measured after flowering was completed) are shown graphically (fig 1A, B and C). In these experiments the plants maintained under short day conditions and given no gibberellin treatment remained healthy and vegetative for at least 6 months. Thereafter, irrespective of night temperature, of all plants grown on short day regimen, 31 % flowered, while the remainder were ultimately discarded because of characteristic physiological or secondary disorders. For this reason the data for numbers of leaves on non-flowering plants (fig 1A) are indicated

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³ The commercial product "Gibrel", the potassium salt of gibberellic acid, was supplied by Merck & Co., Inc., Rahway, New Jersey. It will be referred to as "gibberellin" in this paper.

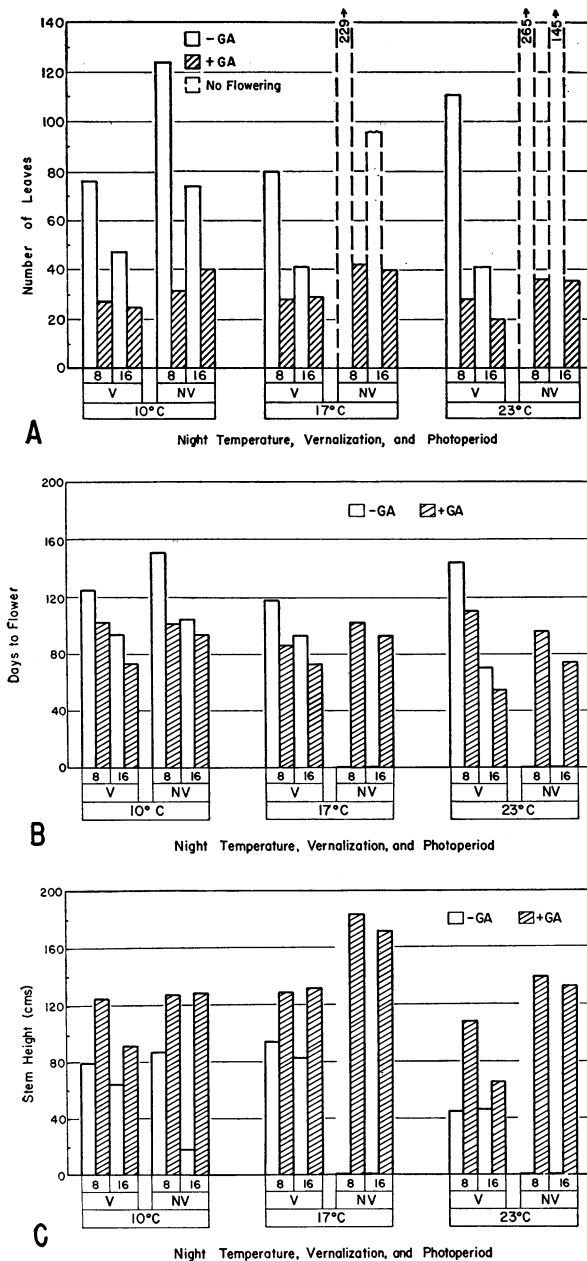


FIG. 1. A) The numbers of leaves per plant below the developing inflorescences, instead of: B) the numbers of days to the appearance of the developing inflorescences, and C) stem heights at the appearance of the developing inflorescence as affected by night temperature, vernalization (V = vernalized, NV = not vernalized), photoperiod (8 or 16 hours) and biweekly foliar applications of gibberellin (50 $\mu\text{g}/\text{plant}$). Broken-lined bars (A) represent number of leaves on plants that remained vegetative until they either flowered or became diseased and were discarded.

by broken-lined bars and no data are given for these treatments on days to flower or stem heights at time of appearance of the inflorescence.

With endive as with lettuce (1, 13) long day (16 hrs) high temperature conditions (17° or 23° C night temperature) after vernalization markedly promote flowering as indicated by decreased leaf numbers and days to flower (fig 1A and B). At night temperatures of 17° and 23° C, under short days 50 % of the vernalized plants eventually developed inflorescences while all non-vernalized plants remained vegetative. Flowering of the vernalized plants grown under short day conditions was sporadic and extended over a period of 3 months. At 10° C, both vernalized and non-vernalized plants flowered, although vernalized plants flowered earlier than non-vernalized plants, indicating that the 10° C night temperature may have partially satisfied the cold requirement. Plants which received gibberellin applied to their foliage, flowered regardless of temperature, photoperiod or vernalization. Gibberellin treated, non-vernalized short day grown plants flowered 8 to 26 days later (depending upon temperature) than did non-gibberellin treated vernalized long day grown plants. Gibberellin application can therefore replace, qualitatively, the requirements of endive both for vernalization and for long day. The effects of vernalization and of gibberellin in accelerating flowering are additive, as has been previously reported (1, 5).

When number of leaves below the developing inflorescence is used as the index of flowering, the ability of gibberellin to replace the effect of vernalization is strikingly clear (fig 1A and table I). Untreated (no gibberellin) vernalized plants on long days produced 41 to 47 leaves before developing an inflorescence, approximately the same number produced by treated but non-vernalized plants on a short day regimen. In contrast, untreated non-vernalized plants

TABLE I.
EFFECT OF FOLIAR APPLICATIONS OF GIBBERELLIN ON FLOWERING IN ENDIVE*

PHOTOPEKIOD HOURS	TREATMENT		NIGHT TEMPERATURE**		
	VERNALIZA- TION 20 DAYS AT 4° C	GIBBERELLIN 50 µg/PLANT BIWEEKLY	10° C	17° C	23° C
<i>Number of days to developing inflorescence</i>					
8	—	+	101***	102	96
16	+	—	93	93	70
<i>Number of leaves below developing inflorescence</i>					
8	—	+	31	42	36
16	+	—	47	41	41

* Data taken from figures 1A and 1B.

** Day temperature: 23° C.

*** Each figure is the average of 6 single plant replications.

both under short and long day conditions did not flower or produced more than 70 leaves before producing an inflorescence.

In the experiment of table II and figure 1C stem height at the time of appearance of the developing inflorescence was measured. This provides an index of the effectiveness of gibberellin in promoting stem growth. A night temperature of 17° C is clearly more favorable to stem growth than night temperatures of 10° C or 23° C (table II). Early flowering, induced by vernalization, was also accompanied by short stems. Applications of gibberellin increased stem height, at all temperatures. The tallest stems, as among all treatments, were produced by gibberellin treated, non-vernalized plants grown on short days. These stems were 2 to 3 times as tall as stems of untreated vernalized plants grown on long days. This response is similar to that of lettuce reported by Bukovac and Wittwer (1).

The results reported above show that application of gibberellin causes flowering of endive under otherwise unfavorable environmental conditions. Further experiments were designed to determine quantitatively the extent to which application of gibberellin can replace the vernalization and long day requirements for flowering of endive. In the first experiment, endive seed was vernalized for 1, 4, 8, 12, 16 or 20 days. Five single plant replications were then placed at a day/night temperature of 20°/14° C under short (8 hrs) or long (16 hrs) photoperiods (figs 2A and B). Control seedlings were started at the same growing temperature on the 1st, 12th or 20th day of vernalization period. After the 2nd true leaf was fully expanded 50 µg of gibberellin were applied biweekly to the growing tip. As in the initial experiment, vernalization markedly hastened flowering of endive

TABLE II.

STEM GROWTH OF ENDIVE AS AFFECTED BY GIBBERELLIN TREATMENT AT 2 PHOTOPERIODS AND 3 NIGHT TEMPERATURES *

PHOTOPERIOD HOURS	TREATMENT		NIGHT TEMPERATURE**		
	VERNALIZA- TION 20 DAYS AT 4° C	GIBBERELLIN 50 µg/PLANT BIWEEKLY	10° C	17° C	23° C
8	—	+	Stem height at appearance of developing inflorescence		
16	+	—			
8	—	+	Stem elongation cm/day		
16	+	—			

* Data interpreted from figure 1C.

** Day temperature: 23° C.

*** Each figure is the average of 6 single plant replications.

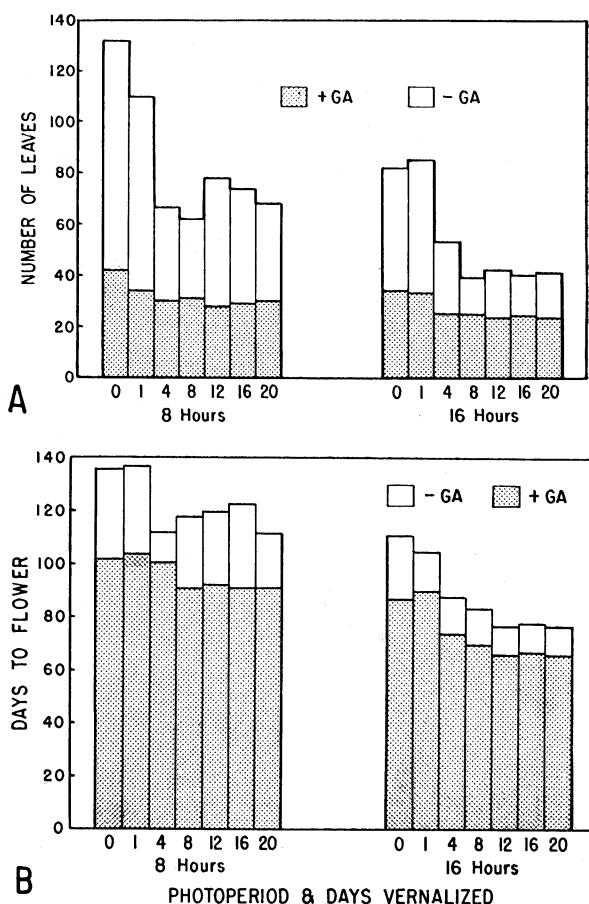


FIG. 2. Interactions of photoperiod (8 or 16 hrs), vernalization (0, 1, 4, 8, 12, 16 or 20 days), and biweekly applications of gibberellin (50 µg/plant) in the flowering of endive: A) The number of leaves below the developing inflorescences. B) The number of days to the appearance of the developing inflorescences.

seedlings grown under long days. The maximal effect of vernalization was achieved after 8 days as judged by the number of leaves developed below the inflorescence. Application of gibberellin again accelerated flowering under both photoperiods, especially in combination with vernalization. It is of interest to compare the behavior of untreated but vernalized long day grown plants with that of treated but non-vernalized short day grown plants. Untreated vernalized (more than 8 days) plants grown under long photoperiod produced approximately 41 leaves before flowering. Treated but non-vernalized plants grown in short days characteristically produced this same number of leaves before flowering (fig 2A). As in the experiment of figure 1, number of days to the appearance of flower parts was slightly greater in gibberellin treated non-vernalized plants grown under short days than in untreated but vernalized plants grown under long days (fig 2B). This was particu-

larly true at the highest (23° C) night temperature. It may be concluded, however, that gibberellin treatment is essentially able to replace completely both the effects of vernalization and of long day.

In the preceding experiments gibberellin was applied to growing endive plants, either vernalized or non-vernalized. In the following experiment the effect of gibberellin treatment of the seed on the flowering response of the plant was investigated. Seeds were vernalized for 20 days at 4° C either in water or in solutions containing 0.1, 1, 10 or 100 ppm of gibberellin. Control seeds were soaked for 2 days at 25° C in water or in gibberellin solutions. The seedlings (16 per treatment) were transferred to a greenhouse at a 20° C constant temperature and 16 hour photoperiod. Plants from gibberellin treated vernalized or non-vernalized seed showed rapid initial growth with somewhat enlarged leaves and slightly elongated stems. Only the plants from vernalized seed, however, produced seedstalks and flowered (15). Untreated, vernalized plants produced an average of 34 leaves before flowering. Vernalized plants grown from seed soaked in concentrations of gibberellin above 1 ppm produced 17 to 25 leaves before flowering, reduction in number of leaves paralleling increase in concentration of gibberellin. Thus seed treatment with gibberellin during vernalization elicited a flowering response essentially similar to that elicited by vernalization followed by biweekly foliar gibberellin treatment of the growing plant (fig 1A and 2A).

DISCUSSION AND CONCLUSIONS

It is evident from the data presented above that gibberellin treatment of growing plants can replace, nearly quantitatively, the effects of vernalization (of the seed) and of long day (on the growing plant) in hastening flowering of endive. This is true whether time to flowering is measured by days or by numbers of leaves produced before appearance of the inflorescence. It is also true, however, that application of gibberellin to growing vegetative endive plants causes seedstalk elongation and promotion of flowering only if such application is repeated frequently. A single application of gibberellin causes only a temporary seedstalk elongation (8). Vernalization, is inductive (causes a persistent after effect) while gibberellin treatment is not.

Although continuing gibberellin treatment is able to replace most of the promotive effects of vernalization and of long day on seedstalk elongation and formation of inflorescences in endive, it is not able to make amends for unfavorable temperatures during growth. The optimum night temperature for stem growth of endive is about 17° C, (table II). This is true both of gibberellin treated and untreated plants although at all night temperatures the growth rate of the gibberellin treated plants is very nearly twice that of the untreated plants. Availability of gibberellin does not therefore appear to be the limiting factor for growth at the less favorable growing temperatures (10° C and 23° C).

SUMMARY

Biweekly applications of gibberellin (50 or 100 µg per plant) were found to replace qualitatively the requirements both for vernalization and for long days for flowering of endive. This was true whether the flowering index used was number of days to the appearance of the developing inflorescence or number of leaves produced before the appearance of flower parts.

Gibberellin treated non-vernalized plants grown on short (8 hour) days were twice as tall at time of appearance of the inflorescence as untreated vernalized plants grown on long days (16 hrs). They nevertheless flowered almost as early, and after producing essentially the same number of leaves.

Gibberellin treatment of growing plants (but not of seed) replaced both the vernalization and long photoperiod requirement for flowering, not only at an optimum temperature (23° day/17° night) but also at lower (10° night) and higher (23° night) temperatures. Gibberellin treatment did not, however, overcome the effects of the less favorable growing temperatures.

ACKNOWLEDGEMENTS

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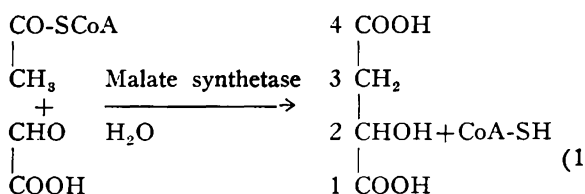
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MALATE SYNTHETASE IN HIGHER PLANTS.¹

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Malate synthetase brings about the condensation of glyoxylate and acetyl CoA to form malate, according to reaction 1.



The enzyme was first discovered in microorganisms by Wong and Ajl (14) and is one of the key enzymes of the glyoxylate cycle described by Kornberg and Krebs (6). In earlier work we have shown that the enzymes of the glyoxylate cycle are present in the endosperm of the germinating castor bean (Kornberg and Beevers 8) and one of these, isocitritase, has been examined in some detail (Carpenter and Beevers 5). In this report a comparable investigation has been carried out on malate synthetase. Its distribution in a variety of plant tissues has been determined and some of the properties of the castor bean enzyme have been investigated. The results reinforce the earlier suggestions that malate synthetase and isocitritase, as components of the glyoxylate cycle are an essential part of the machinery by which fats are converted to carbohydrate.

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² Recipient of Fulbright Travel Grant. Permanent Address: Biological Institute, Faculty of Science, Nagoya University.

MATERIALS AND METHODS

PLANT MATERIALS Germination of the castor beans and the preparation of crude extracts (castor bean preparation) were carried out by methods described previously (8). Other seeds were germinated in a similar fashion on vermiculite and grown in the dark at 30° C for the stated periods. Mature parts were cut from plants growing in the greenhouse.

Lactobacillus arabinosus, adapted to malate, was grown by the method of Nossal (9) as modified by Stiller (11). Lyophilized cells were stored at –15° C.

Special chemicals Sodium glyoxylate was obtained from Mann Biochemicals. Acetate-1-C¹⁴ and acetic-1-C¹⁴ anhydride at a rated specific activity of 1 millicurie per millimole were supplied by Nuclear of Chicago.

Acetyl-1-C¹⁴-CoA was prepared from the anhydride by the method of Simon and Shemin (10). All measurements of radioactivity were made on BaCO₃ in a windowless gas flow counter and the results are corrected for background and self absorption. Combustions to determine the C¹⁴ content of substrates were carried out according to Stutz and Burris (13).

Aceto-CoA-kinase was prepared from yeast using Berg's (3) method to the stage of the first ammonium sulfate precipitation. It was stored in the frozen condition and maintained its activity over several months. The kinase was shown to have a low but detectable malate synthetase activity. In the results of those experiments in which it was used, these blank values (150–250 cpm) have been subtracted.

All enzyme incubations were carried out at 30° C.